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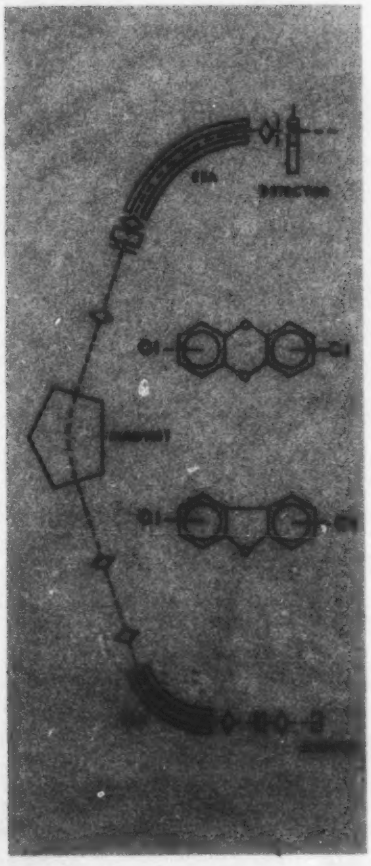

Reference Method for the Determination of Polychlorinated Dibenzo-*para*-dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) in Pulp and Paper Mill Effluents

Report EPS 1/RM/19
February 1992

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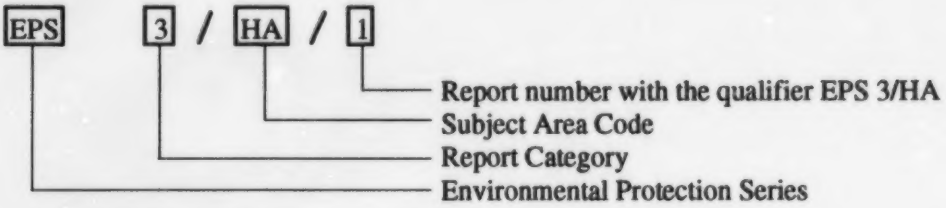

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Foreword

The analytical methodology described in this report has been developed in support of the Canadian Environmental Protection Act *and is used to identify and quantify trace amounts of polychlorinated dibenzo-*para*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in pulp and paper mill effluents. The method detection limit depends on sample matrix characteristics and can be as low as 2 to 20 pg/L (parts per quadrillion) for PCDD and PCDF congeners.*

*The following procedures are described in detail: sample collection; extraction; cleanup; chromatographic separation; and identification and quantification of tetra- through octa- CDD/CDF congeners, including 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF).*

Application of this analytical methodology for compliance testing requires strict adherence to all critical elements in this method. Throughout this report, such critical elements are identified by bold type. Any deviation from the critical elements of this method may invalidate the test results and must be approved in writing by Environment Canada before testing. If deviations are made without prior approval, the validity of the test results shall be determined by Environment Canada on a case-by-case basis.

Avant-propos

La méthode d'analyse décrite dans le présent document a été élaborée pour l'application de la Loi canadienne sur la protection de l'environnement; *elle sert à identifier et à doser les polychlorodibenzo-*para*-dioxines (PCDD) et les polychlorodibenzofuranes (PCDF) présents à l'état de traces dans les effluents des usines de pâtes et papiers. La limite de détection de la méthode dépend de la matrice de l'échantillon et peut être aussi faible que de 2 à 20 pg/L (parties par 10^{-12}) pour les congénères de PCDD et de PCDF.*

*On décrit en détail la marche à suivre pour le prélèvement, l'extraction et l'épuration des échantillons ainsi que pour la séparation par chromatographie, l'identification et le dosage des tétra- à octa-CCD/CDF, y compris les congénères spécifiques 2,3,7,8-tétrachlorodibenzo-*para*-dioxine (2,3,7,8-TCDD) et 2,3,7,8-tétrachlorodibenzofurane (2,3,7,8-TCDF).*

Pour les essais de conformité, il faut suivre rigoureusement toutes les étapes critiques de la méthode d'analyse, qui sont indiquées en caractères gras dans le présent document. Toute modification touchant ces éléments critiques risque d'invalider les résultats obtenus et doit être approuvée par écrit par Environnement Canada avant les essais. Si des modifications sont apportées sans autorisation préalable, la validité des essais sera déterminée, cas par cas, par Environnement Canada.

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Section 1

Summary

A 1-L sample of pulp effluent is initially separated into solid and liquid phases by suction filtering the sample through a glass fibre filter. The solid phase is air-dried overnight before undergoing a Soxhlet extraction with toluene. The liquid phase is extracted with dichloromethane in a separatory funnel. Before filtration, the sample is spiked with a surrogate standard mixture of isotopically-labelled polychlorinated dibenzo-*para*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners.

The two extracts are combined, concentrated, and then passed through a series of columns which remove, by reaction and/or selective adsorption, the bulk of the organic matrix co-extracted with PCDD/PCDF. One resulting fraction is concentrated to a known small volume for analysis.

Qualitative/quantitative analysis for PCDD/PCDF is performed with a high-resolution gas chromatograph/ high-resolution mass spectrometer/ computerized data system (HRGC/ HRMS/DS). Two characteristic ions are selectively monitored for each PCDD/PCDF congener group. Analyte identification is confirmed when target ions are detected in the correct abundance ratio within established retention time windows. Quantification is based on the use of surrogates (isotopically-labelled compounds added before sample workup) as internal standards. Two other isotopically-labelled standards, added to sample extracts immediately before analysis, serve as recovery standards for the quantification of surrogate recovery.

Section 2

Contamination and Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines that could lead to elevated detection limits and/or loss of ability to detect PCDDs/PCDFs that may be present. Reagents should be of the highest available purity. In some cases, reagents may require further purification before use.

Proper cleaning of glassware is extremely important. Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware filled with a detergent solution can also be performed as an aid to cleaning.

It shall be demonstrated that all materials used in the analysis are free from interferences by running reference matrix blanks initially and with each sample set. High purity water is used as the reference matrix blank.

Interferences co-extracted from samples will vary considerably from source to source depending upon the exact nature of the sample matrix. Interfering compounds may be present at concentrations several orders of magnitude higher than any PCDDs/PCDFs that may be present. Interfering co-extractants must therefore be eliminated or reduced to the maximum extent practicable in order to ensure reliable quantification of trace amounts of PCDDs/PCDFs. The cleanup procedures described in this report can effectively remove many potential interferents.

Despite rigorous cleanup procedures, matrix interference will still be a possibility. If detection limits are seriously affected by excessive background (non-discrete interference), the sample extract will have to be re-processed using alternative cleanup techniques (Creaser and Haddad, 1989; NCASI Method, June 1990).

Section 3

Safety

Although the degree of toxicity has not been precisely established for all compounds used or analyzed by this method, each one should be treated as a potential health hazard and exposure to any of them should be minimized as much as possible (EC and HWC, 1990).

All work related to analyzing PCDDs and PCDFs, including preparation, handling and storage of all samples and standards, should be carried out in a specially designed laboratory. Such a facility should include the following design/operational specifications:

- restricted access;
- sufficient ventilation;
- negative pressure relative to surrounding areas;
- all exhaust air ducts routed to a common, scrubbed outlet;
- full operational capability on auxiliary power if a commercial power failure occurs;
- an independent backup air supply system designed to operate if the building's common air supply system shuts down;
- capacity to visually monitor ventilation performance;
- distinctive audio and visual alarm systems to alert all building personnel to potentially hazardous conditions.

Personnel in the lab must wear protective clothing at all times, consisting of safety glasses, disposable coveralls, disposable foot coverings (optional), and disposable gloves. Other personal protective devices, such as face masks and cartridge or canister respirators, should be available in the lab in the event of a minor spill. Common laboratory safety equipment and facilities, such as safety showers, first aid kits, eye wash stations, and fire extinguishers must be easily accessible.

Only experienced and specially-trained personnel who are fully aware of the hazards associated with the chemicals under study, should be involved in these analyses. Laboratory safety policies must be established, covering such topics as safe and secure handling and storage of PCDDs and PCDFs, housekeeping, spill containment and cleanup, and containment of solid and liquid wastes. Copies of these policies must be provided to lab personnel, and they must be adhered to strictly.

Section 4

Apparatus and Reagents

4.1 Equipment and Supplies

Extraction Apparatus: 200-mL Soxhlet body (Dean-Stark moisture trap optional), condenser, 45 × 125-mm glass or cellulose thimble, 500-mL boiling flask, and a temperature-controlled heating mantle

Evaporative Concentrator: Büchi R-110 rotary evaporator or equivalent

Balance: Top-loading macro balance, capacity to 1600 g, readability to 0.01 g, for weighing samples and reagents

Pipets: Electronic or mechanical pipets with capacities from 10 to 1000 µL

Vacuum Oven: Range to 225°C, for conditioning carbon, silica, reagent-coated silica, sodium sulphate, and glass wool

Tube Furnace: Lindberg or equivalent, for activating alumina at 350°C

Pumps: Pressure/Vacuum with vacuum to 85 kPa (25 in. Hg) for rotary evaporation and suction filtration

Filters: Gelman A/E or equivalent, 11 cm diameter

Weighing Paper

Forceps, Spatulas, Scoops

Ultrasonic Bath

Dessicators

Automatic Glassware Washing Equipment

GC/MS/DS: See Section 6

4.2 Glassware

Beakers: Assorted volumes from 150 to 2000 mL

Buchner Funnels: 12 cm diameter

Filtration Flasks: 2 L

Separatory Funnels: 2 L

Boiling Flasks: 250 and 500 mL

Erlenmeyer Flasks: 500 mL with glass stopper

Powder Funnels: 7 cm diameter

Graduated Cylinders: 10, 50, 100, 250 mL

Pasteur Pipets: 22 cm, disposable

Petri Dishes: 14 cm diameter

Glass Column: Minimum 1 L capacity, Teflon stopcock, for solvent washing of silica, sodium sulphate, and glass wool

Cleanup Columns: Acid/Base, Alumina (see Figure 4)

Alumina Conditioning Column: See Figure 1

Measuring Pipets: 5.0 mL and 10.0 mL, graduated at 0.1 mL intervals, for preparing PCDD/PCDF standard mixtures

Sample Extract Vials: 1 mL capacity, internally-tapered, screw cap vials with Teflon-faced septa

Vials: Assorted capacities from 1.5 to 40 mL, amber glass, screw cap with Teflon-faced septa

4.3 Reagents

Standards: Certified native PCDD/PCDF standard solutions, carbon-13-labelled standards, window defining mixtures and column performance mixtures are available from Cambridge Isotope Laboratories, Woburn, MA.

Solvents: All solvents are distilled-in-glass quality. Those required include hexane, toluene, dichloromethane, and acetone.

Gases: Ultra-high purity grade nitrogen and helium

Water: An in-house supply of high purity distilled or deionized water is used (Millipore Milli-Q Water System or equivalent system using a carbon filtration stage).

Others: Silica (Bio-Sil A, 100 to 200 mesh) and basic alumina (AG 10, 100 to 200 mesh) are available from Bio-Rad Laboratories, Richmond, CA. Suitable equivalents may be available from other sources. AX-21 carbon is available from Anderson Development Company, Adrian, MI. All other reagents, including sulphuric acid, sodium hydroxide, silver nitrate, and sodium sulphate are A.C.S. reagent grade or better.

4.4 Glassware and Material Preparation

Glassware: All reusable glassware must be scrupulously cleaned as soon as possible after use. Glassware is sequentially rinsed with the last solvent used, followed by hexane and acetone. This is followed by washing with hot detergent solution and sequential rinsing with hot water, deionized water, and three portions each of acetone, hexane, and dichloromethane. For severely contaminated glassware, treatment in an ultrasonic bath filled with detergent solution is often beneficial. If a brush is used to scrub glassware, great care must be taken to ensure that glass surfaces are not scratched. Glassware is either air-dried or dried in an oven, and then stored in a contaminant-free area.

A glassware proofing sample must be analyzed before actual test samples are processed. Each piece of glassware to be used with test samples, including Soxhlet bodies and condensers, flasks, funnels, columns, vials and rotary evaporators, must be rinsed with three portions each of hexane and dichloromethane. All rinses are combined into one sample, which is spiked with surrogates, cleaned up (optional) **and analyzed by GC/MS to determine potential interferents and any amounts of PCDD/PCDF that may be present. Sample analysis may proceed only if any PCDD/PCDF present is below maximum allowable levels (see Section 8).**

Glass Wool: Compress a quantity of glass wool into a large glass column (1 L capacity or larger) and wash sequentially with hexane and dichloromethane. The volume of solvent used for each wash should be twice the estimated volume of glass wool in the column. Transfer the washed glass wool into a large beaker. Loosely cover the mouth of the beaker with hexane and dichloromethane-rinsed aluminum foil, allow the glass wool to air dry in a fume hood, and then condition overnight at 225°C in a vented oven. Store in a clean, wide-mouth, glass-stoppered bottle.

Sodium Sulphate: Wash the granular, anhydrous sodium sulphate in the same column used for preparing glass wool. Sequentially wash the sodium sulphate twice with hexane and twice with dichloromethane. The volume of solvent used for each washing should be twice the estimated volume of sodium sulphate in the column. Transfer to a large beaker, cover the mouth loosely with solvent-rinsed aluminum foil, and oven dry at 50°C for a minimum of one hour before conditioning overnight at 225°C. Store in a dessicator in a clean, screw-capped bottle fitted with a Teflon-lined cap.

Silica: Transfer approximately 350 g of Bio-Sil A (enough silica for 35 cleanup columns) to the large glass column used for preparing glass wool and sodium sulphate. Sequentially wash with hexane and dichloromethane as previously described. Oven dry the silica at 50°C for a minimum of one hour in a beaker loosely covered with solvent-rinsed aluminum foil, then condition at 225°C for a minimum of four hours. Store the clean silica in a dessicator, in a clean, screw-capped glass bottle fitted with a Teflon-lined cap.

44% (w/w) H_2SO_4 on Silica: Add 78.6 g of concentrated H_2SO_4 in a stepwise manner (5 mL at a time), to 100 g of freshly conditioned silica in a 500-mL glass-stoppered Erlenmeyer flask. After each addition, shake the flask vigorously to remove all clumps. Store in the stoppered flask. This amount of material is sufficient for 40 cleanup columns. Preparation of larger batches is not recommended. **(Caution:** This material has all the properties of concentrated H_2SO_4. Handle with care.)

33% (w/w) of 1 M NaOH on Silica: Add 24.6 g of a 1 M NaOH solution, in a stepwise manner, to 50 g of freshly-conditioned silica in a glass-stoppered Erlenmeyer flask. After each addition, shake the flask to remove all clumps. Store in a screw-capped bottle fitted with a Teflon-lined cap.

10% (w/w) $AgNO_3$ on Silica: Dissolve 5.6 g of silver nitrate in 21.5 mL of deionized water. Add this solution, in a stepwise manner, to 50 g of freshly-conditioned silica in a glass-stoppered Erlenmeyer flask. Between additions, shake the flask until a uniformly-coated, free-flowing powder is produced. When all silver nitrate has been added, allow the material to stand for approximately 30 minutes, cover the mouth of the flask with solvent-rinsed aluminum foil, and place in an oven at 30°C. Over a five-hour period, gradually raise the oven temperature to 120°C, and continue to condition overnight at this temperature. Cool to room temperature and immediately transfer to an amber glass, screw-capped bottle fitted with a Teflon-lined cap. Minimize exposure of this material to light. Store in a desiccator until use.

5% AX-21 Carbon/Silica: Wash 7 g of AX-21 carbon powder by suspending in 100 mL of methanol, then vacuum filter through a glass fibre filter (Gelman A/E or equivalent) fitted in a Buchner funnel. Follow with two 100-mL methanol rinses and continue suction until methanol flow stops completely. Dry the washed AX-21 carbon in a vacuum oven for several hours at room temperature and then maintain at 130°C for a minimum of 72 hours.

Combine 5.0 g of prepared AX-21 carbon with 95 g of prepared silica gel in a wide-mouth bottle with a Teflon-lined screw cap. Blend by shaking until a uniform colour is achieved. Activate the blended AX-21

carbon/silica at 130°C for a minimum of 24 hours, then cap and store in a dessicator. Reactivation of the carbon/silica may be required after several weeks of storage.

Basic Alumina: Weigh out 2 to 3 g more alumina than is required (2.5 g/sample) for the number of samples to be batch-processed at one time. Add the alumina to the conditioning column, shown in Figure 1, and wash with hexane, then dichloromethane (two portions each). The volume of solvent used for each wash should be two to three times the estimated volume of alumina in the conditioning column. After draining, insert a pre-cleaned glass wool plug into the column to immobilize the alumina. Drain as much solvent as possible from the wet alumina by applying suction at the end of the column, then place the column in the tube furnace. Connect the glass-jointed end of the column to a cylinder of pre-purified nitrogen. With the furnace off, purge the alumina with nitrogen at 200 to 400 mL/min. for approximately 30 minutes. While maintaining the nitrogen purge, condition the alumina at 350°C for a minimum of two hours. Conditioned alumina should be used immediately after removal from the tube furnace. Do not store for later use. (**Caution:** Hot glass.)

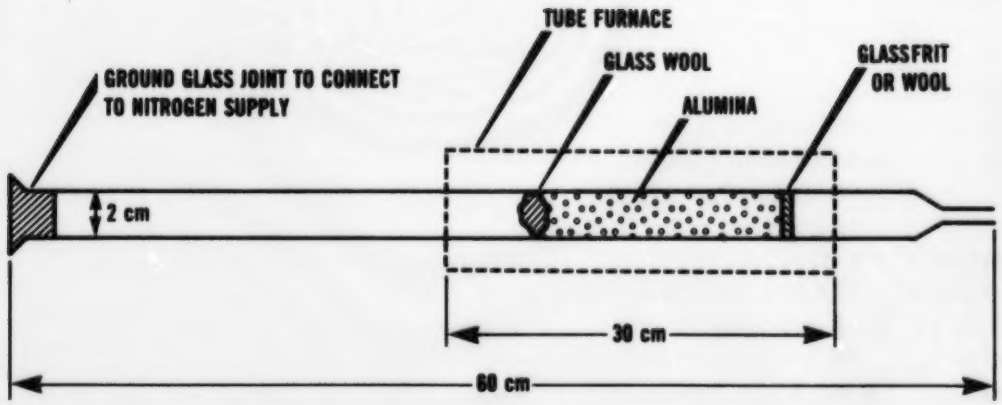

Figure 1 Alumina Conditioning Column

Section 5

Sample Handling and Workup Procedures

5.1 Sample Handling and Custody

Upon arrival at the laboratory, samples must be inspected immediately for their physical condition and to ensure proper labelling. Inform the client of any potential problem concerning the integrity of samples. After logging and labelling with laboratory code numbers, samples should be processed as soon as possible. **Any sample-tracking report sheets submitted with samples are completed and signed by authorized lab personnel and retained for auditing. The laboratory's analysis-tracking documents must also be available for auditing.**

Samples must be maintained at 1 to 5°C from the time of receipt until extraction. (**Caution:** Prevent from freezing.)

5.2 Method Performance Tests

Before sample analysis, the laboratory must demonstrate the ability to achieve acceptable precision and accuracy by conducting method performance tests.

Three matrix blanks (1 L of high purity water), spiked with known amounts of native standards and labelled surrogates, must be prepared, processed and analyzed according to the procedures used for actual samples. The native congener spiking solution must contain all 2,3,7,8-substituted dioxin and furan congeners at concentrations equal to those shown for the CS2 calibration standard in Table 4. The composition of the surrogate spiking solution is shown in Table 1. The required spike volume is 50 µL of each solution. Both the native and surrogate standard solutions are diluted with acetone immediately before spiking, as described in Subsection 5.3.

For each of the three tests, recovery for each of the native PCDD/PCDF congeners (corrected for surrogate recovery) must be within the range of 80 to 120% of the spiked value (i.e., accuracy of ± 20%). All labelled surrogate recoveries must be within the range of 40 to 120%.

No field sample shall be processed until the method performance tests yield acceptable results. These tests must be repeated whenever extraction or cleanup procedures are modified in any way, whenever a different reagent lot number is used, or if performance tests have not been conducted in the preceding three-month period.

5.3 Sample Preparation

For each batch of 1-L samples processed, prepare a fresh dilution of the PCDD/PCDF surrogate solution as shown in Table 1 by adding 50 µL of this stock solution to 950 µL of acetone (1×20 dilution) for each sample being prepared.

Weigh the nominal 1-L sample, in its bottle, to the nearest gram on a top-loading balance. Sample weight is determined later by subtracting the weight of the dried bottle. Net sample weight must be between 900 g and 1100 g. Within these limits, weight is converted to volume by assuming a sample density of 1.0 g/mL.

Table 1 Composition of Surrogate Solution

Surrogate	Stock Solution in Toluene (pg/μL)	Spike Solution in Acetone (pg/μL)
$^{13}C_{12}$-2,3,7,8-TCDD	20	1
$^{13}C_{12}$-2,3,7,8-TCDF	20	1
$^{13}C_{12}$-1,2,3,7,8-P_5CDD	20	1
$^{13}C_{12}$-1,2,3,7,8-P_5CDF	20	1
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD	20	1
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF	20	1
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD	20	1
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF	20	1
$^{13}C_{12}$-OCDD	40	2

$^{13}C_{12}$-OCDF is not used because it may interfere with OCDD analysis

Spike the sample (in the bottle) with 1.0 mL of the freshly diluted surrogate solution (Table 1). Carefully shake the bottle several times or mix the solution with a magnetic stirrer during the next hour to allow the surrogates to equilibrate within the raw sample matrix.

After one hour, suction filter the sample through a pre-weighed Gelman A/E glass fibre filter into a 2-L filtration flask. Complete the transfer with several portions of deionized water to remove any remaining particulate. Retain the sample bottle for later use as described in Subsection 5.4.1. Continue suction until the flow of filtrate stops completely. Transfer the filter and any particulate still adhering to the walls of the Buchner funnel to a Petri dish and allow the solids to dry in a dessicator. Weigh the dried filter containing the solid portion. This fraction of the sample is extracted in a Soxhlet apparatus. The filtrate is extracted in a separatory funnel.

5.4 Sample Extraction

Extraction procedures are shown in Figure 2. Detailed descriptions are provided in the following subsections.

5.4.1 Extraction of Filtrate

Quantitatively transfer the filtrate from the flask to a clean 2-L separatory funnel using three rinses of deionized water. Add three, 60-mL rinses of dichloromethane to the retained empty sample bottle, shaking each rinse in the bottle before transferring to the separatory funnel. Extract the filtrate with these rinses by shaking the separatory funnel vigorously for two minutes, releasing pressure buildup as required. Allow the sample to sit for at least ten minutes to ensure separation of the aqueous and organic layers. Drain the organic layer into a 500-mL round bottom flask. Persistent emulsions may be drained off into a clean, large beaker and broken using mechanical means, such as passage through loosely packed glass wool.

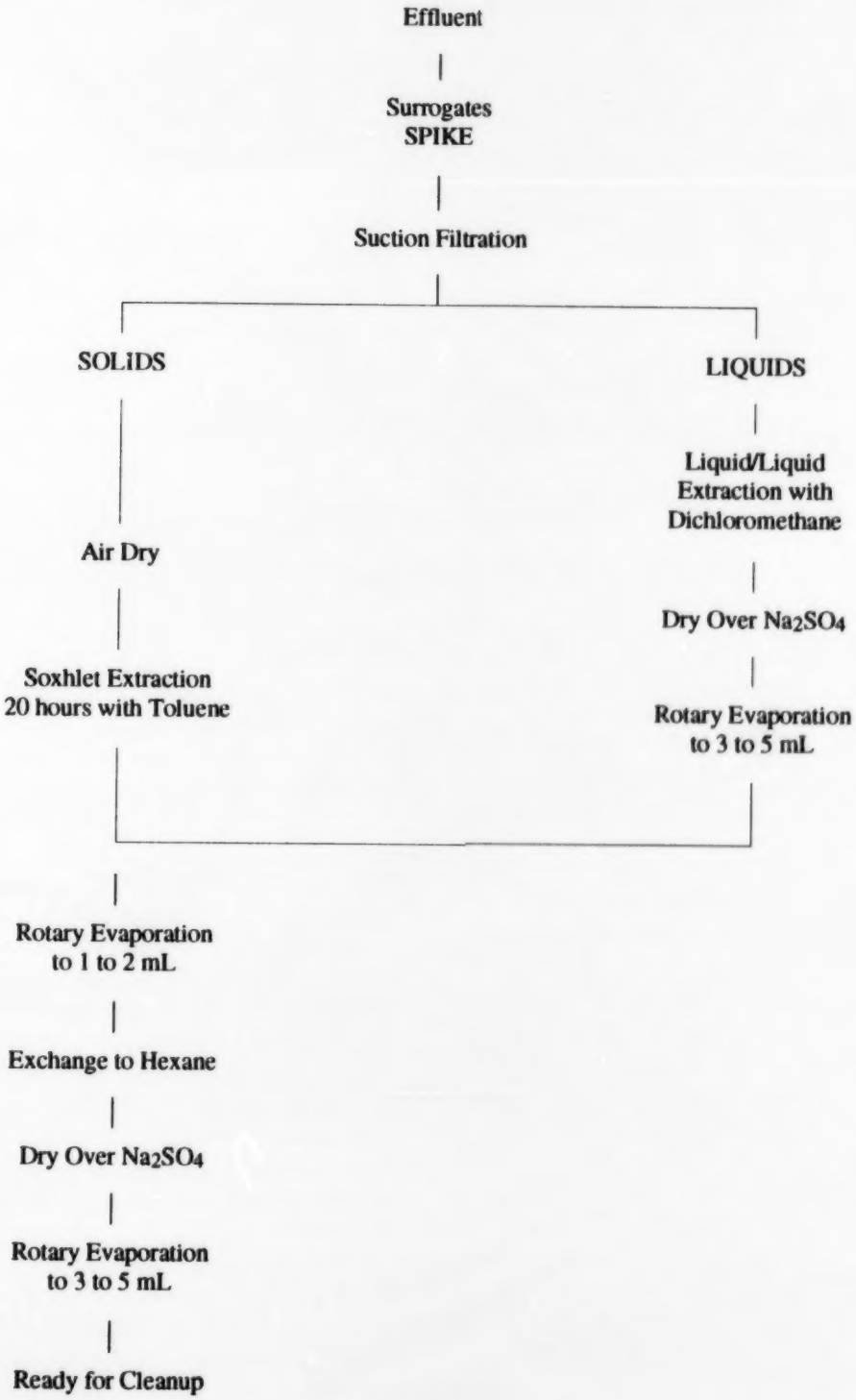

Figure 2 Extraction Schematic for Pulp and Paper Mill Effluents

Repeat these extraction procedures two more times using 100 mL of dichloromethane each time. Rinse the separatory funnel with an additional 30 mL of dichloromethane. If any water or emulsion is observed in the collection flask at this point, the extract must be passed through a bed of dichloromethane-rinsed sodium sulphate held in a powder funnel positioned over a second 500-mL flask. Concentrate the combined extracts and rinses to 3 to 5 mL by rotary evaporation at 30°C before combining with the extract from the particulate fraction of the sample.

5.4.2 Extraction of Particulate Matter

Place a pre-extracted thimble in a clean glass beaker and transfer the filter, containing the dried particulate, into the thimble. Rinse any small amounts of solids remaining in the Petri dish into the thimble with toluene, then cover the sample with a layer of clean glass wool. Place the thimble into the Soxhlet body. Fill the flask with approximately 350 mL of toluene. Connect the flask to the Soxhlet body. Rinse the beaker three times with toluene and add the rinsings into the Soxhlet body. Connect the Soxhlet to the water-cooled condenser and wrap the Soxhlet body with aluminum foil for heat insulation. Reflux the sample at a rate of three to four cycles per hour.

After a minimum of 16 hours of extraction, rinse the inner surface of the condenser with toluene and allow the apparatus to cool. Siphon the solvent from the Soxhlet body into the extract flask. Rinse the Soxhlet body three times with toluene, siphoning each rinse into the flask.

Add the concentrated filtrate extract to the particulate extract with three hexane rinsings of the flask. Concentrate the combined sample to 1 to 2 mL by rotary evaporation at 72°C or lower. Exchange the solvent to hexane by adding approximately 100 mL of hexane to the sample flask and repeating the concentration step.

Dry the sample by passing it through hexane-rinsed sodium sulphate held in a powder funnel positioned over a 250-mL boiling flask. Complete the transfer by rinsing first the extraction flask, then the sodium sulphate, with three 5-mL portions of hexane. Concentrate the sample to 3 to 5 mL by rotary evaporation at 30°C. The sample is now ready for cleanup.

5.5 Sample Cleanup

The analyst may use the following cleanup procedures, or any other procedures that yield a sample extract fraction of sufficient quality to satisfy all method performance criteria for target analyte determination.

The basic cleanup procedures recommended for sample extracts are shown in Figure 3. Cleanup columns required in this method are shown in Figure 4 (Lamparski and Nestrick, 1980; EC, 1989).

Acid/Base/Silver Nitrate /Silica Column: Plug the tip of the column with glass wool and add the following in the order specified: 1.5 g of 10% $AgNO_3$/silica (bottom layer); 1 g of silica; 2 g of the 33% 1 M NaOH/silica; 1 g of silica; 4 g of 44% H_2SO_4/silica; 2 g of silica; and approximately 1 g of sodium sulphate to top off the column. After each reagent has been added, gently tap the column to ensure even layering.

Pre-wash the prepared column with 30 mL of 2% dichloromethane in hexane (v/v). Just as the solvent reaches the top of the sodium sulphate layer, place a 250-mL flask under the column. Via Pasteur pipet, transfer the concentrated raw extract onto the column,

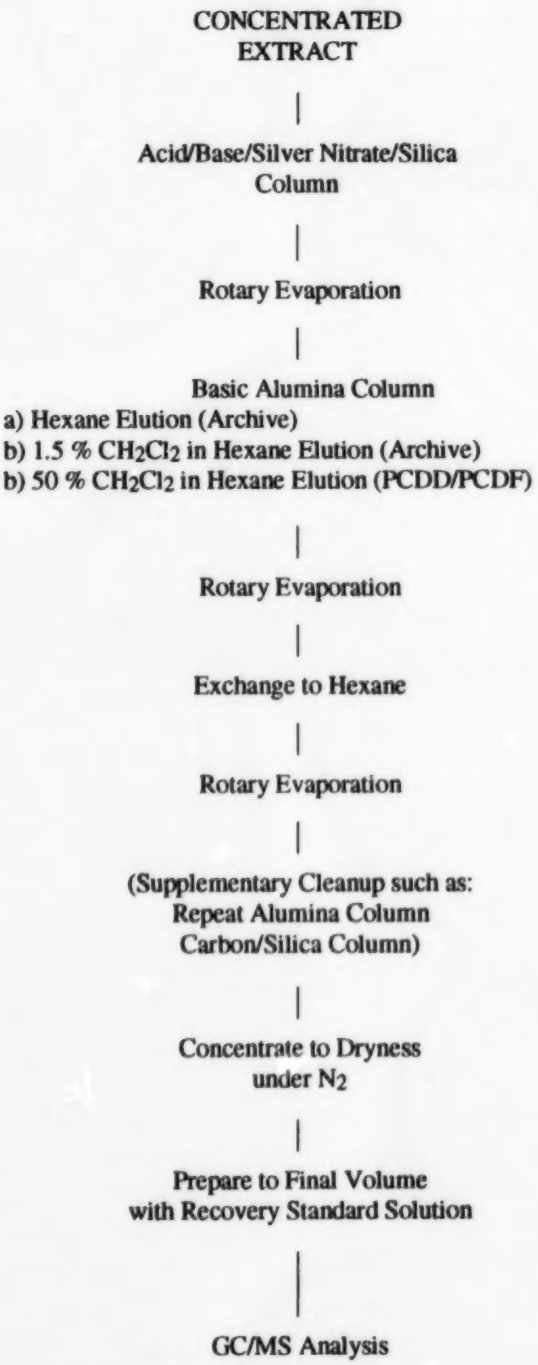

Figure 3 Cleanup and Analysis Schematic for PCDDs/PCDFs in Pulp and Paper Mill Effluents

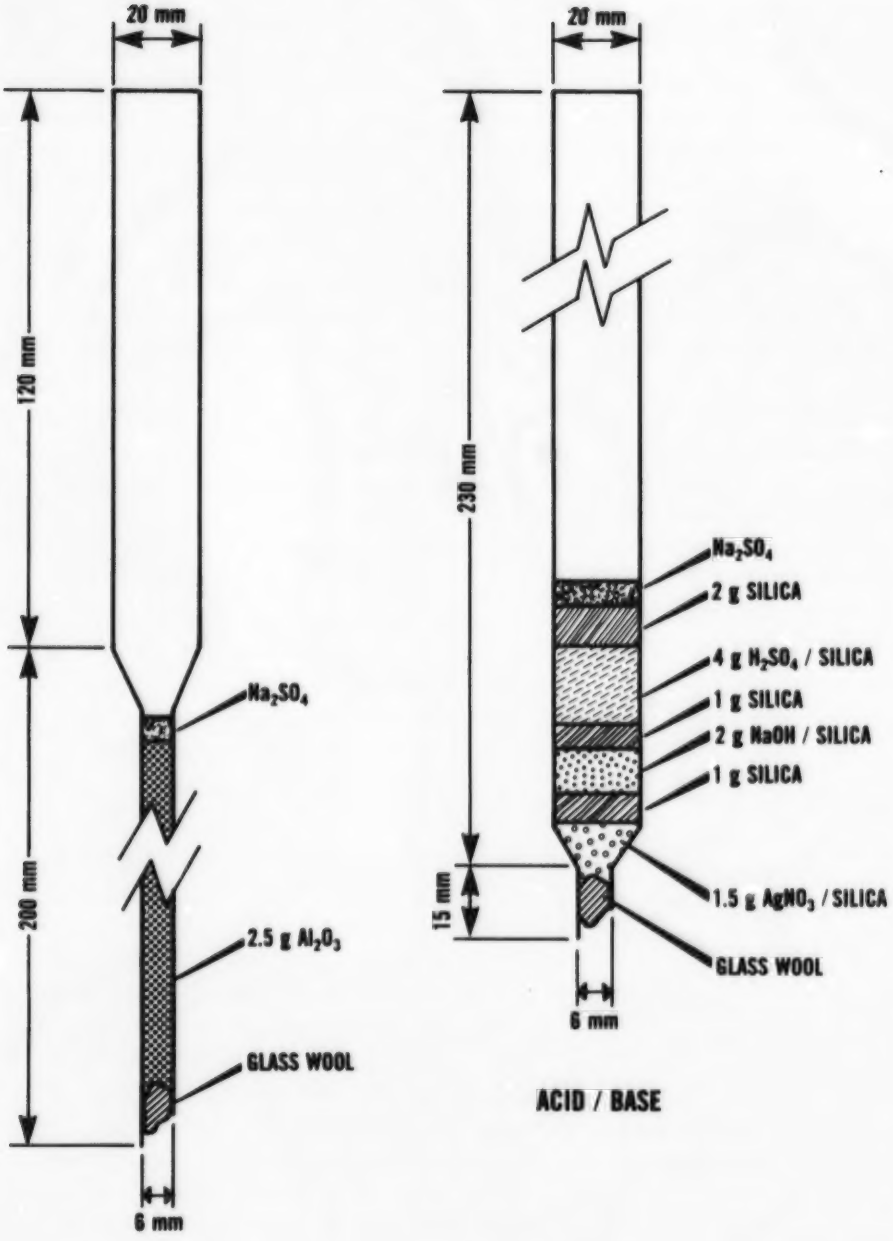

Figure 4 Cleanup Columns

followed by three 5-mL 2% dichloromethane in hexane (v/v) rinsings of the sample flask, using the same pipet each time.

When the third rinse has drained to the top of the sodium sulphate layer, pour an additional 50 mL of 2% dichloromethane in hexane (v/v) into the column. When the solvent has drained, assess the column for saturation of the acid/silica layer and the silver nitrate/silica layer. This is indicated by an appearance of colour throughout the reagent layer.

Saturation of the acid/silica layer suggests that the sample extract (in hexane) should be washed with concentrated sulphuric acid in a separatory funnel, repeating the washes (maximum of four washings) with fresh acid until no colour is observed in the acid layer. Wash the extract sequentially with deionized water, 1 M NaOH and a final wash with deionized water. Dry the extract by passing it through sodium sulphate, as previously described.

Saturation of the silver nitrate/silica layer requires passage of the concentrated sample extract through an additional column containing 2.5 g of 10% silver nitrate/silica. Elute the sample extract through the pre-washed column with 30 mL of 2% dichloromethane in hexane.

Add 100 mL of hexane to the column eluate and concentrate to 1 to 2 mL by rotary evaporation at 30°C.

Alumina Column Chromatography: Plug the tip of the alumina column with glass wool and add 2.5 g of freshly prepared basic alumina (see Subsection 4.4). Top off the column with 0.5 cm of sodium sulphate. Add 15 mL of hexane to pre-wash the column. When the hexane has drained just to the top of the sodium sulphate layer, immediately place a 250-mL boiling flask, labelled Fraction 1, under the column tip. Via Pasteur pipet, transfer the concentrated extract from the acid/base/silver nitrate/silica column onto the alumina column, followed, just as the sample extract reaches the sodium sulphate layer, by three 5-mL hexane rinsings of the sample flask. Add an additional 30 mL of hexane (2 x 15 mL) to the column just as the flask rinsings reach the top of the sodium sulphate layer. When this solvent has drained to the top of the sodium sulphate layer, add 20 mL of freshly prepared 1.5% dichloromethane in hexane to the column. This combined fraction (Fraction 1) is archived. When the solvent level in the column again just reaches the top of the sodium sulphate layer, exchange the Fraction 1 flask for a 250-mL flask, labelled Fraction 2.

Add 30 mL of 50% dichloromethane in hexane (v/v) to the column and allow the column to drain completely. This fraction contains the PCDDs/PCDFs. Concentrate this fraction to 1 to 2 mL and exchange the solvent to hexane by adding approximately 100 mL of hexane to the sample flask and repeating the concentration step.

Prepare another alumina column (optional) as just described. Then, following the same procedure, apply the sample (Fraction 2) to the column and collect Fraction 1 in the flask containing that eluate from the first column. Fraction 2 is also collected in its original flask. Concentrate both fractions to approximately 1 mL by rotary evaporation at 30°C. Transfer Fraction 1 to a 1.5 mL amber vial and archive. This fraction may be assessed for PCDDs/PCDFs if poor surrogate recovery is observed in Fraction 2.

Fraction 2, containing the PCDDs/PCDFs, is transferred via Pasteur pipet to a 1-mL conical sample vial (as specified in Section 4.2) and concentrated under a gentle stream

of pre-purified nitrogen. Complete the sample transfer using the same pipet to take up three, 0.5-mL hexane rinses of the flask, transferring each rinse into the vial. Concentrate the extract to a small volume (approximately 100 µL) and store the sample vial at 5°C or lower in a refrigerator until analysis.

Immediately before GC/MS analysis, blow down the sample just to dryness under a gentle stream of pre-purified nitrogen. Add 20 µL of the recovery standard solution, containing 50 pg/µL each of $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD in toluene, to the sample vial. Sonicate the capped vial for one minute or allow to sit for a minimum of one hour before analysis.

AX-21 Carbon/Silica Column (optional): Prepare a 8 × 260 mm glass column by cutting both ends off a disposable 10-mL serological pipet. Pack the column in the following sequence: glass wool plug; 1 g of activated carbon/silica; 1 cm of silica; glass wool plug. Pre-rinse the column with 5 mL of 1:1 cyclohexane/dichloromethane with the silica plug at the bottom. Invert the column and rinse with a second 5 mL of 1:1 cyclohexane/dichloromethane. The sample, in approximately 1 mL of hexane, is applied onto the column with the silica plug at the top. Complete the transfer of sample onto the column using 2 × 2 mL of 1:1 cyclohexane/dichloromethane to rinse the sample flask. Elute with 6 mL of 1:1 cyclohexane/dichloromethane, followed by 20 mL of 75:20:5 dichloromethane/ methanol/toluene. Discard all eluates up to this point. Invert the column so that the silica plug is at the bottom, and elute with 30 mL of toluene. Collect the eluate in a 250-mL flask. Concentrate the sample using rotary evaporation at 72°C and transfer to a reacti-vial as previously described.

Section 6

Gas Chromatographic/Mass Spectrometric Analysis

6.1 Instrumentation

Requirements for the gas chromatograph (GC) are: that it exhibits isothermal temperature stability of ±0.2 C°, or better, over its specified range of operation; that it have capability to accomodate a minimum of three temperature ramps; and that it be configured for use with a capillary column. The capillary column is directly coupled to a high-resolution, double-focussing mass spectrometer (MS) of any geometry. The MS is operator-programmed for time-sequenced acquisition of selected MS data for each congener group. The MS is operated in the electron impact (EI) and selected ion monitoring (SIM) modes.

6.2 Gas Chromatograph (GC) Parameters

Optimum operating conditions must be achieved for both the gas chromatographic separation and the subsequent mass spectrometric analysis of the separated constituents.

To achieve acceptable gas chromatographic separation on a 60 metre DB-5 (polymethyl [5% phenyl] silicone), or equivalent column (see Subsection 6.3), the following set of parameters can be used as a starting point: Injector temperature: 300°C for split-splitless, or ambient for on-column; Interface temperature: 290°C; Temperature program: 1) Initial temperature of 100°C for split-splitless or 70°C for on-column and hold for 1 minute; 2) 100 (or 70) to 200°C at 40 C°min^{-1}; 3) 200 to 235°C at 3 C°min^{-1}; 4) hold at 235°C for 10 min.; 5) 235 to 310°C at 8 C°min^{-1}; and 6) hold at 310°C for 15 min.

Optimum settings for GC parameters and appropriate retention time windows for time-sequenced SIM mode analysis of PCDDs/PCDFs on a 60 metre DB-5 column are established from the analysis of Window Defining Mixtures containing the first and last eluting congeners within each homologue group of analytes. The order of elution, as shown in Table 2, is such that five retention windows can be defined, corresponding to the five levels of chlorine substitution (4 Cl to 8 Cl), without any overlap. Under optimum conditions, the interval between the latest eluting TCDD and TCDF congeners and the earliest eluting P_5CDF congeners is no more than 0.1 minute. Parameter settings that produce a 2,3,7,8-TCDD retention time of 25 minutes or more will normally represent conditions under which the 4 Cl/5 Cl gap is optimized and the chromatographic performance criterion described in the following subsection can be satisfied.

6.3 Isomer-Specific Separation

Using helium as carrier gas at an appropriate linear velocity, and with an appropriate oven temperature program, a 60 metre DB-5 column (0.25 mm ID, 0.25 μm film thickness) can adequately separate 2,3,7,8-TCDD from neighbouring isomers 1,2,3,7-/1,2,3,8- and 1,2,3,9-TCDD. In addition, this column easily separates to baseline both hepta-CDD isomers and the four hepta-CDF isomers. However, on a DB-5 column, 2,3,7,8-TCDF cannot be resolved from its neighbouring isomers

Table 2 Elution Order of PCDD/PCDF Window Defining Mixture on a 60-metre DB-5 Column

Homologue	First Eluting Isomer	Last Eluting Isomer	Retention Time Window* (min.)
TCDD	1,3,6,8-	1,2,8,9-	25.0 to 29.7
TCDF	1,3,6,8-	1,2,8,9-	23.4 to 29.7
P_5CDD	1,2,4,6,8/ 1,2,4,7,9-	1,2,3,8,9-	31.5 to 34.0
P_5CDF	1,2,4,6,8/ 1,3,4,6,8-	1,2,3,8,9-	30.0 to 34.2
H_6CDD	1,2,4,6,7,9/ 1,2,4,6,8,9-	1,2,3,4,6,7-	35.6 to 37.3
H_6CDF	1,2,3,4,6,8-	1,2,3,4,8,9-	35.1 to 37.8
H_7CDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-	39.9 to 40.7
H_7CDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-	39.5 to 41.3
OCDD			47.0
OCDF			47.3

* On the basis of temperature program given in subsection 6.2.

(i.e., 1,2,4,9-, 2,3,4,8- and 2,3,4,6-). **In order to accurately quantify any 2,3,7,8-TCDF that may be present in a sample, analysis on a second column is required. Use of a second column is only mandatory when results from the DB-5 run indicate that the concentration of 2,3,7,8-TCDF may equal or exceed the regulatory level.** On a 30 metre DB-225 column, 2,3,7,8-TCDF can be resolved from its neighbouring 2,3,4,7- and 1,2,3,9- isomers.

A 60 metre DB-Dioxin column can also be used as an alternative for 2,3,7,8-TCDD and 2,3,7,8-TCDF analysis because this column is capable of separating 2,3,7,8-TCDD from neighbouring isomers 1,2,4,6-/1,2,4,9- and 1,2,3,7-/1,2,6,8-TCDD, and 2,3,7,8-TCDF from neighbouring isomers 2,3,4,7- and 2,3,4,8-TCDF. However, it cannot be used effectively for homologue analysis.

It is important to recognize that most of the 2,3,7,8-substituted congeners cannot be uniquely identified on a DB-5 column, even under optimum conditions. For this (and any other) column, all of the congeners that would be required to verify the chromatographic separation of individual 2,3,7,8-substituted penta- and hexa-CDD/CDF congeners from their nearest neighbours are not commercially available at the present time. Therefore, amounts reported for target penta- and hexa-CDD/CDF congeners represent

maximum possible concentrations. Results for these target analytes must be properly flagged to warn about the possibility of other co-eluting isomers.

Acceptable chromatographic performance, illustrated in Figure 5, is determined by analyzing a Column Performance Mixture containing either 2,3,7,8-TCDD or 2,3,7,8-TCDF, and its nearest neighbours, at equal concentrations. On a 60 metre DB-5 column, the height of the valley between the most closely eluting peaks must not exceed 25% of the 2,3,7,8-TCDD peak height. On a 30 metre DB-225 column, neither valley must exceed 30% of the 2,3,7,8-TCDF peak height. Daily verification of acceptable chromatographic resolution is required.

If the appropriate valley criterion is not met, it should be evident whether or not minor adjustment of chromatographic parameters alone will be sufficient to bring performance into specification. If that is not the case, it is usually possible to restore performance without replacing the column. **Whenever the column is disconnected at either end while trying to restore chromatographic performance, or for any other reason, all other GC and MS performance specifications (see Subsections 6.2 and 6.4) must be re-established or re-verified before proceeding with sample analysis, and recalibration is required (see Subsection 6.6).**

Other well-documented and commercially available GC columns, which are not described in this method, may also be used if the user can demonstrate that the appropriate valley criterion has been met.

Window settings and column performance can be assessed in a single run by combining the appropriate mixtures. By comparing the native or labelled 2,3,7,8-TCDD retention time in subsequent standard and sample runs to that obtained in the combined window/performance run, the fine cutoff point between the tetra and penta windows can be adjusted accurately to compensate for any retention time drift that might occur over the course of a day.

6.4 Mass Spectrometer (MS) Parameters

The mass spectrometer is operated under electron impact (EI) conditions. It is recommended that the electron energy be optimized for detection of PCDD/PCDF molecular cluster ions. This value is expected to be in the range of 28 to 40 eV.

Accurate mass measurements must be made to the third decimal place. In the present method, the mass spectrometer is operated at a static resolution of 10 000 (10% valley). An example of 10 000 resolution is given in Figure 6. Reference perflurokerosene (PFK) is leaked via the reference inlet system and a few scans are acquired to establish a normal calibration file against a PFK reference table for the spectra to be recorded subsequently. **Tune the instrument at a minimum of 10 000 resolving power (10% valley) at m/z 304.9824 and, using peak matching, verify that m/z 380.9760 of PFK falls within 5 ppm of the exact value. Acceptable resolution must be confirmed immediately following each series of injections related to the application of this Reference Method. Verification at appropriate intermediate points of lengthy injection series is highly recommended. Hard copies of these measurements (peak shape and peak width) must be available for auditing purposes.** The PFK leak is continued throughout the sample data

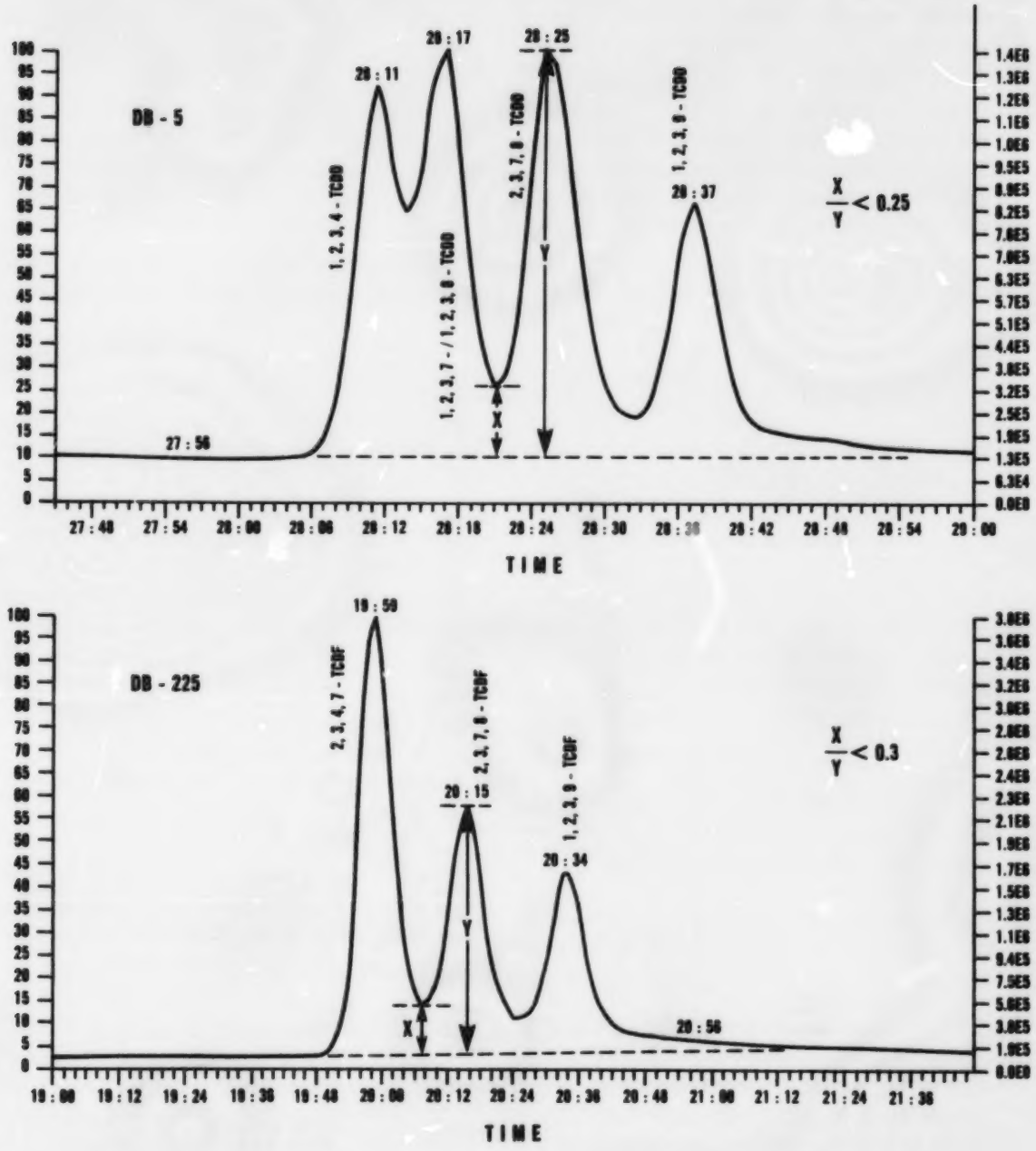

Figure 5 Acceptable Chromatographic Separation for 2,3,7,8-TCDD and 2,3,7,8-TCDF

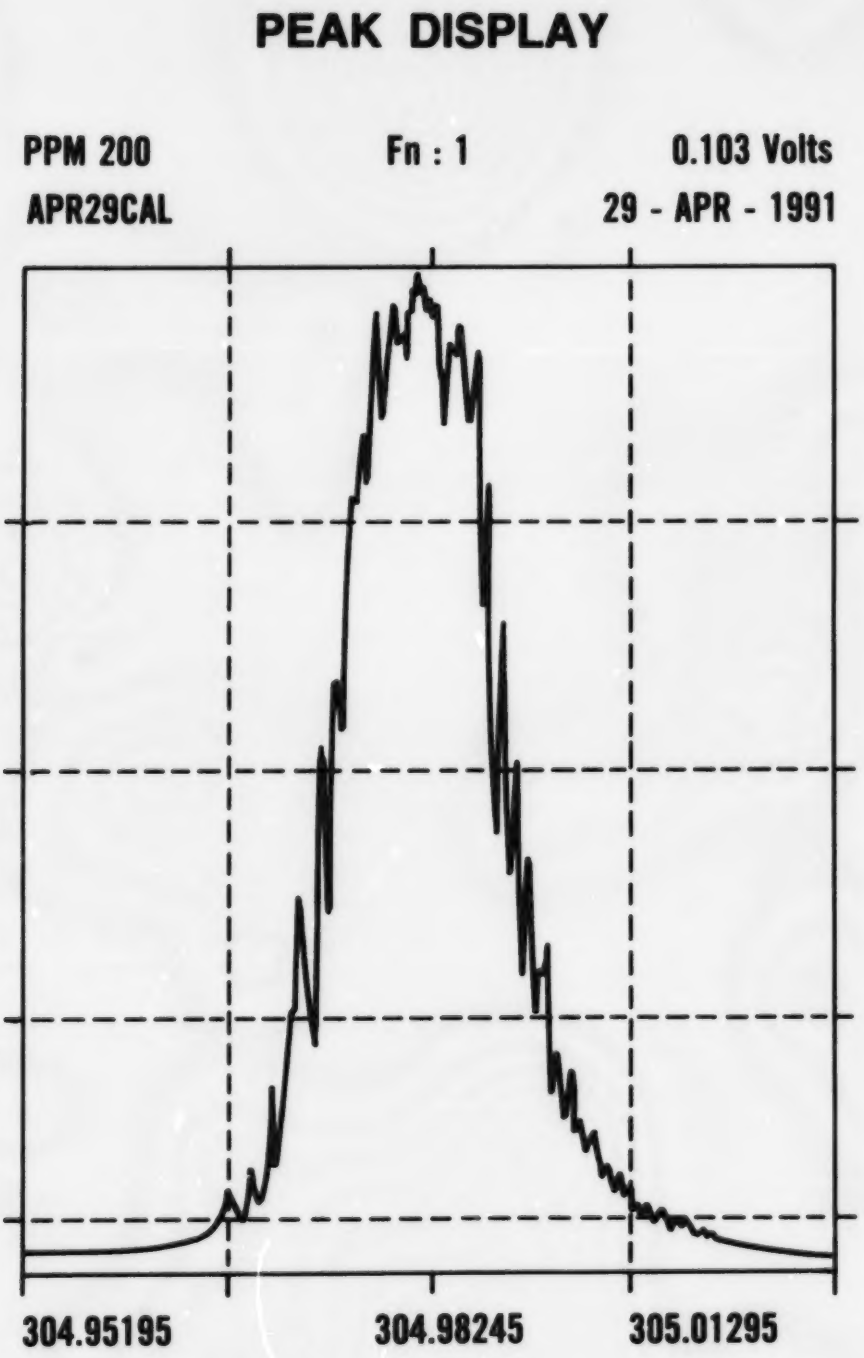

Figure 6 Peak Display for PFK m/z 304.9824 at 10 000 Resolution

acquisition cycle to ensure that the MS remains locked onto the exact masses of all analytically significant ions.

The level of reference compound (PFK) that is allowed to enter the ion source should be monitored and adjusted to ensure that the amplitude of the most abundant lock-mass ion does not exceed 10% of full-scale deflection for the detector parameters used for the analysis. This will allow for more effective monitoring of any sensitivity changes that may occur during analysis.

Each lock-mass must be monitored and its intensity must not vary by more than 10% throughout its respective window. Variations of more than 10% may indicate the presence of co-eluting interferences that may substantially reduce the sensitivity of the instrument (U.S. EPA, 1990). At this stage, re-injection of the sample will not correct the problem; the only viable alternative is further cleanup of the extract, until lock-mass remains constant within 10% throughout the entire duration of its window. **Lock-mass traces must be available for possible audit.**

At 10 000 or better resolution, and with a total scan time of approximately one second for each group of ions specified in Table 3, inject 1 to 2 μL of PCDD/PCDF calibration standard CS3 (Table 4) into the GC/MS system. The isotope abundance ratio for each and every native and surrogate analyte must agree with the corresponding theoretical ratio within the control limits given in Table 3. If any ratio is outside its control limits, a problem clearly exists which must be identified and corrected before proceeding further. If the problem is found to be related to MS parameter settings, any changes at this stage are likely to alter MS resolution, which must therefore be measured again once acceptable ion abundance ratios have been achieved.

Calibration standard CS1 is used to assess instrumental detection limits. **At 10 000 resolution, injection of 0.25 pg of TCDD and TCDF must result in a peak response which is at least five times the background noise level (i.e., S/N ≥ 5) for each of the four monitored ions.** If these levels cannot be detected, then instrument parameters are to be adjusted accordingly, while maintaining acceptable resolution. In other words, the instrument is not in acceptable operating condition until all detection and measurement criteria are satisfied simultaneously.

6.5 Criteria for Analyte Identification

Sample components are identified as PCDDs/PCDFs if their GC/MS data satisfy the following criteria:

(a) **Peak responses for each of the two selected molecular cluster ions must be at least three times the background noise level** (i.e., S/N ≥ 3, see Subsection 6.7 for a definition of noise).

(b) **Chlorine isotope ratio for the two molecular cluster ions must be within 15 % of the correct ratio** (see Table 3).

(c) **Peak maxima for both quantification ions must coincide within two seconds.**

(d) **Reported sample concentrations of PCDF must be flagged with an "E" if any peak recorded in the chlorinated diphenyl ether channel maximizes within two seconds of the retention time of an apparent PCDF congener, and response for the ether ion**

Table 3 Selected Ion Masses, Ion Type and Control Limits

Window Number	Compound	Quantification Ions (m/z)		Ion Type	Control Limits for Isotope Ratio
		1st	2nd		
	TCDF	303.9016	305.8987	M/M+2	0.65 - 0.89
	$^{13}C_{12}$-TCDF	315.9419	317.9389	M/M+2	0.65 - 0.89
1	TCDD	319.8965	321.8936	M/M+2	0.65 - 0.89
	$^{13}C_{12}$-TCDD	331.9368	333.9339	M/M+2	0.65 - 0.89
	H_6CDPE*	375.8364		M+2	
	PFK	316.9824		Lock	
	P_5CDF	339.8597	341.8567	M+2/M+4	1.32 - 1.78
	$^{13}C_{12}$-P_5CDF	351.9000	353.8970	M+2/M+4	1.32 - 1.78
2	P_5CDD	355.8546	357.8516	M+2/M+4	1.32 - 1.78
	$^{13}C_{12}$-P_5CDD	367.8949	369.8919	M+2/M+4	1.32 - 1.78
	H_7CDPE*	409.7974		M+2	
	PFK	366.9792		Lock	
	H_6CDF	373.8208	375.8178	M+2/M+4	1.05 - 1.43
	$^{13}C_{12}$-H_6CDF	383.8639	385.8610	M/M+2	0.43 - 0.59
3	H_6CDD	389.8157	391.8127	M+2/M+4	1.05 - 1.43
	$^{13}C_{12}$-H_6CDD	401.8559	403.8529	M+2/M+4	1.05 - 1.43
	O_8CDPE*	445.7555		M+4	
	PFK	380.9760		Lock	
	H_7CDF	407.7818	409.7789	M+2/M+4	0.88 - 1.20
	$^{13}C_{12}$-H_7CDF	419.8220	421.8191	M+2/M+4	0.88 - 1.20
4	H_7CDD	423.7766	425.7737	M+2/M+4	0.88 - 1.20
	$^{13}C_{12}$-H_7CDD	435.8169	437.8140	M+2/M+4	0.88 - 1.20
	N_9CDPE*	479.7165		M+4	
	PFK	430.9728		Lock	
	OCDF	441.7428	443.7398	M+2/M+4	0.76 - 1.02
	OCDD	457.7378	459.7348	M+2/M+4	0.76 - 1.02
5	$^{13}C_{12}$-OCDD	469.7780	471.7750	M+2/M+4	0.76 - 1.02
	D_{10}CDPE*	513.6775		M+4	0.76 - 1.02
	PFK	454.9728		Lock	

* Response of the chlorinated diphenyl ether must be absent for PCDF determination as it has a similar isotope ratio.

Table 4 Composition of PCDD/PCDF Calibration Solutions

PCDD/PCDFs Standard	pg/μL (in toluene)			
	CS1[a]	CS2	CS3[b]	CS4
Native standards[c, d]				
2,3,7,8-TCDD	0.25	1	5	25
2,3,7,8-TCDF	0.25	1	5	25
1,2,3,7,8-P_5CDD	0.50	2	10	50
1,2,3,7,8-P_5CDF	0.50	2	10	50
2,3,4,7,8-P_5CDF	0.50	2	10	50
1,2,3,4,7,8-H_6CDD	0.50	2	10	50
1,2,3,6,7,8-H_6CDD	0.50	2	10	50
1,2,3,7,8,9-H_6CDD	0.50	2	10	50
1,2,3,4,7,8-H_6CDF	0.50	2	10	50
1,2,3,6,7,8-H_6CDF	0.50	2	10	50
2,3,4,6,7,8-H_6CDF	0.50	2	10	50
1,2,3,7,8,9-H_6CDF	0.50	2	10	50
1,2,3,4,6,7,8-H_7CDD	0.50	2	10	50
1,2,3,4,6,7,8-H_7CDF	0.50	2	10	50
1,2,3,4,7,8,9-H_7CDF	0.50	2	10	50
OCDD	1	4	20	100
OCDF	1	4	20	100
Surrogates				
$^{13}C_{12}$-2,3,7,8-TCDD	50	50	50	50
$^{13}C_{12}$-2,3,7,8-TCDF	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-P_5CDD	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-P_5CDF	50	50	50	50
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD	50	50	50	50
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF	50	50	50	50
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD	50	50	50	50
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF	50	50	50	50
$^{13}C_{12}$-OCDD	100	100	100	100
Recovery Standards				
$^{13}C_{12}$-1,2,3,4-TCDD[e]	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8,9-H_6CDD[f]	50	50	50	50

[a] also used to assess detection limits; [b] used daily to verify calibration and abundance ratios; [c] penta- and hepta-CDF, hexa-CDD/CDF isomers listed in order of elution on a DB-5 column; [d] a solution of native congeners at CS2 concentrations is used as a spiking solution for method performance tests (see Subsection 5.2); [e] retention time marker and recovery standard for tetra- and penta-CDD/CDF; [f] retention time marker and recovery standard for hexa- and hepta-CDD/CDF and OCDD.

exceeds 5% of the sum of the peak areas for the two monitored furan ions.

(e) **Sample components are identified as 2,3,7,8-substituted congeners if the following applicable criterion is also satisfied:**

1) In the case of a congener for which a labelled analogue is present in the surrogate spiking mixture, all native and surrogate ion peak maxima must be coincident within three seconds.

2) In the case of a congener for which a labelled analogue is not present in the surrogate spiking mixture, peak maxima for both quantification ions must be coincident with each other within two seconds, and both peaks must be within three seconds of the expected retention time for that congener. Expected retention time is determined on the basis of known relative retention times for these congeners.

6.6 Quantification

An internal standard method is used to quantify PCDDs/PCDFs. It relies upon consistent linearity of MS response over time and over the calibration range represented by the standard solutions defined in Table 4. This internal standard method is easily integrated into an automated routine for data quantification.

Internal standard quantification is based on the use of Relative Response Factors (RRF). For native standards, the RRF is the ratio of analyte response factor to the response factor of the corresponding labelled surrogate (internal standard). These RRFs remain unchanged over the range of concentration for which MS response is linear. Using these RRFs, along with native and surrogate responses from the sample run, recovery-corrected concentrations of PCDDs/PCDFs are calculated directly. Surrogate recoveries are calculated separately and reported, as these values reflect the overall quality of the concentration data being reported.

Relative response factors for the native standard (RRF_n) and for the surrogate standard (RRF_s) are calculated using the following equations:

$$RRF_n = \frac{A_c \cdot C_{sc}}{A_{sc} \cdot C_c}$$

and

$$RRF_s = \frac{A_{sc} \cdot C_{rc}}{A_{rc} \cdot C_{sc}}$$

where:

RRF_n = relative response factor, native standard to surrogate standard;

RRF_s = relative response factor, surrogate standard to recovery standard;

A_c = quantification ion (single or both ions) peak area for native standard;

A_{sc} = quantification ion (single or both ions) peak area for the appropriate surrogate standard;

A_{rc} = quantification ion (single or both ions) peak area for $^{13}C_{12}$-1,2,3,4-TCDD or $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD;

C_c = concentration of the native standard (pg/μL);

C_{sc} = concentration of the appropriate surrogate standard (pg/μL);

C_{rc} = concentration of $^{13}C_{12}$-1,2,3,4-TCDD or $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD (pg/μL).

Using the RRFs, sample concentrations of PCDDs/PCDFs (C) and surrogate standard recoveries (%R) can be calculated as follows:

$$C(X) = \frac{\left(\sum_{k=1}^{n} A_k\right) \cdot Q_{ss}}{A_{ss} \cdot RRF_n \cdot V}$$

and

$$\%R(X) = \frac{A_{ss} \cdot Q_{rs} \cdot 100}{A_{rs} \cdot Q_{ss} \cdot RRF_s}$$

Where:

C(X) = recovery-corrected concentration of analyte X (pg/L);

A_k = quantification ion (single or both ions) peak area for the "k"th homologous isomer of analyte X (n=1 for isomer-specific analysis);

Q_{ss} = amount of surrogate standard X added to the sample (pg);

A_{ss} = quantification ion (single or both ions) peak area for surrogate standard X in sample extract;

V = sample size (L);

%R(X)= percent recovery of surrogate standard X;

Q_{rs} = amount of $^{13}C_{12}$-1,2,3,4-TCDD (recovery standard for tetra- and penta-CDD/CDF) or $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD (recovery standard for hexa- and hepta-CDD/CDF and OCDD) in sample extract (pg);

A_{rs} = quantification ion (single or both ions) peak area for $^{13}C_{12}$-1,2,3,4-TCDD or $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD in sample extract.

For homologues represented by more than one isomer in the calibration standard solutions, the "homologue-average" RRF is used to quantify all target analytes that are not 2,3,7,8-substituted congeners.

The calibration data from which RRFs are calculated must be of good and definable quality. For initial calibration, RRFs are calculated by analyzing the series of four calibration standard solutions, the compositions of which are given in Table 4. The Relative Standard Deviation (RSD) of the four RRFs must be less than 15% for both 2,3,7,8-TCDD and 2,3,7,8-TCDF, and less than 20% for all other native analytes. This value effectively corresponds to a response linearity criterion. **If this criterion is met, then the calibration is successful, and the mean RRF values are used for quantification of subsequent target analyte data. The established calibration curve must be verified by analyzing the calibration verification standard (CS3) at least once during every 12-hour period in which sample analysis occurs** (see Section 8).

6.7 Limit of Detection

The Method Detection Limit (MDL) for PCDD/PCDF analysis is defined as the minimum concentration of analyte in the sample that will produce clearly defined

peaks with an acceptable chlorine isotope ratio, and with a signal-to-noise ratio equal to three for the quantification ion exhibiting the poorer signal-to-noise ratio. Variables, such as sample matrix, sample size, final extract volume, injection volume used in analysis, surrogate recovery, GC column performance, chromatographic parameters, electronic noise, and instrument sensitivity may directly influence the MDL.

Reported MDL must be corrected for surrogate recovery and is calculated as follows:

$$MDL = \frac{3 \cdot N \cdot {}^{A}/_{H} \cdot Q_{ss}}{A_{ss} \cdot RRF_n \cdot V}$$

where:

N = estimated sum of electronic and chemical (matrix) noise expressed as peak height (single or both ions);

A/H = area/height ratio for the surrogate standard peak;

Q_{ss} = amount of surrogate standard added (pg);

A_{ss} = surrogate peak area (single or both ions);

RRF_n = relative response factor, as defined in the preceding subsection;

V = sample size (L).

Average noise amplitude can be determined manually, as shown in Figure 7. The area/height ratio observed for the corresponding surrogate ion peak(s) in the sample data is used to convert noise amplitude to an area value.

The noise for each homologue group must be determined from the actual sample chromatograms. If a quantification ion channel contains one or more large peaks that prevent observation of the noise, a portion of the chromatogram should be re-scaled so that the noise level can be assessed.

When detection of any of the seventeen 2,3,7,8-substituted congeners is rejected solely due to an incorrect isotope ratio, reporting requirements are as follows: 1) in Figure 8, Part I, report the result as NDR in the pg/L column; 2) calculate a concentration value for the congener in the same manner as if the ratio had been within specification and report this value, in brackets, in the Detection Limit column; 3) in Figure 8, Part II, bracket the ion(s) used for quantification; and 4) report the actual isotope ratio, in brackets, in the adjacent column. The bracketed concentration value reported in Part I is not to be included in the corresponding homologue total.

On the basis of these procedures, assuming adequate surrogate recoveries, MDL target ranges for pulp mill effluents are listed in Table 5.

6.8 Level of Quantification (LOQ)

The LOQ is defined as the lowest level above which quantitative results may be obtained with a specified degree of confidence. A single LOQ concentration value for the determination of 2,3,7,8-TCDD and 2,3,7,8-TCDF in pulp mill effluents by this method was established from statistical evaluation of data generated in an interlaboratory study. Ten commercial, industrial, and governmental laboratories participated in the study, which involved analysis of eight replicate portions of two pulp mill effluent samples containing low and near-detection-limit levels of

2,3,7,8-TCDD and 2,3,7,8-TCDF. Following screening of data for statistical validity, pooled standard deviations of the measured concentrations were calculated. **By defining the LOQ as ten times the standard deviation of measured concentrations at near-detection-limit levels (Keith *et al.*, 1983), and using a pooled estimate of standard deviation from the interlaboratory study, the LOQ concentration value for 2,3,7,8-TCDD and 2,3,7,8-TCDF was established as 15 ppq. Method precision at or near the LOQ concentration is estimated to be ± 5 ppq at the 99% confidence level.**

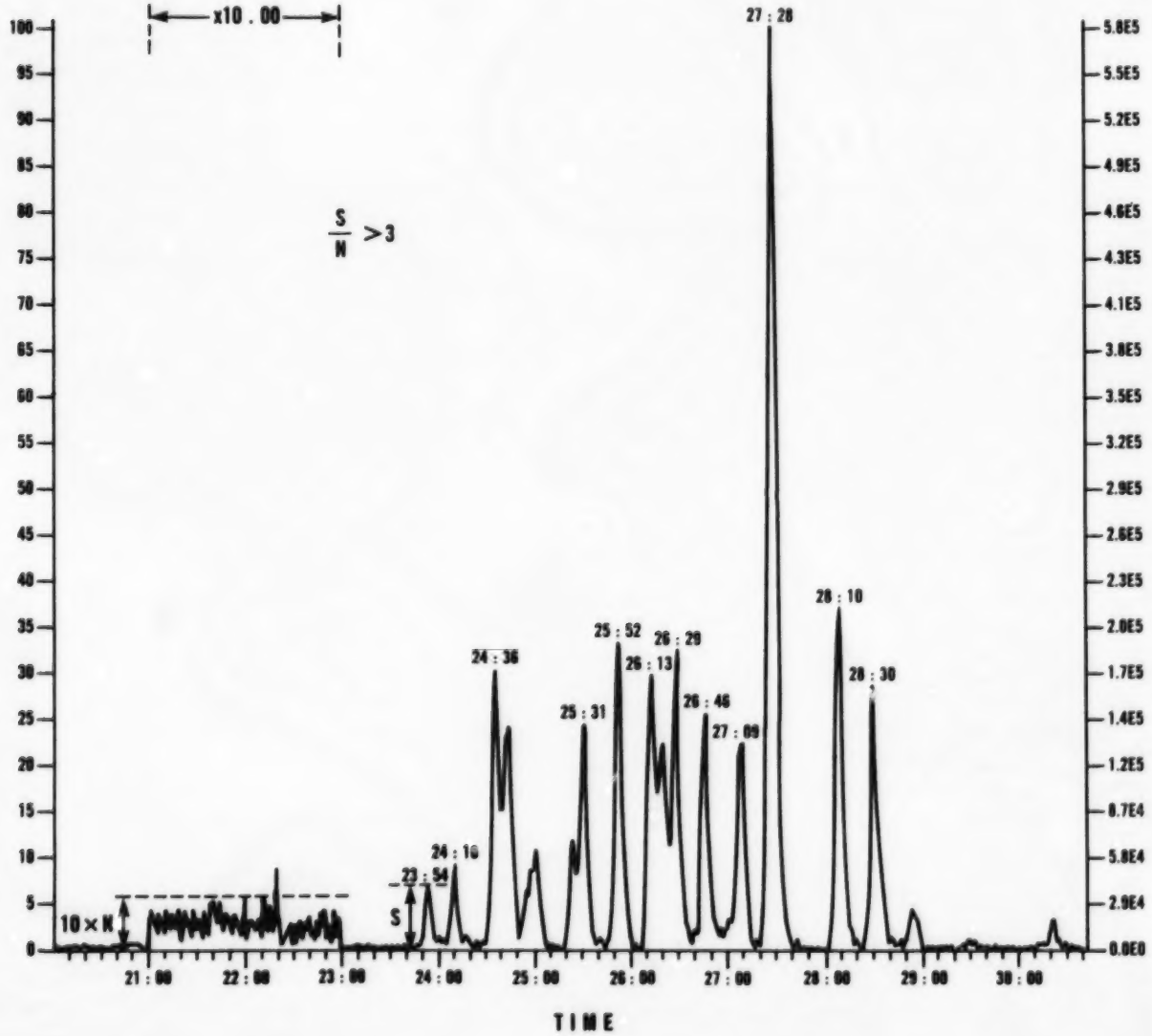

Figure 7 Noise Determination

Effluent Sample Data Sheet-Part I

Lab: _____ Filtered Solid Weight (Dry): _____
Mill: _____ GC Column & MS: _____
Sample ID: _____ Initial Calibration Date: _____
Sampling Date: _____ Analysis Date: _____
Sample Volume: _____ File: _____
Approved by: _____

Congener	pg/L (ppq)	DL	Homologue	pg/L (ppq)	DL	NP
2,3,7,8-TCDD			TCDD			
1,2,3,7,8-P_5CDD*			P_5CDD			
1,2,3,4,7,8-H_6CDD*			H_6CDD			
1,2,3,6,7,8-H_6CDD*			H_7CDD			
1,2,3,7,8,9-H_6CDD*			OCDD			
1,2,3,4,6,7,8-H_7CDD						
OCDD			Total PCDD			
2,3,7,8-TCDF						
1,2,3,7,8-P_5CDF*			TCDF			
2,3,4,7,8-P_5CDF*			P_5CDF			
1,2,3,4,7,8-H_6CDF*			H_6CDF			
1,2,3,6,7,8-H_6CDF*			H_7CDF			
2,3,4,6,7,8-H_6CDF*			OCDF			
1,2,3,7,8,9-H_6CDF*						
1,2,3,4,6,7,8-H_7CDF			Total PCDF			
1,2,3,4,7,8,9-H_7CDF						
OCDF						

Surrogate	Amount Added pg	% Recovery
$^{13}C_{12}$-2,3,7,8-TCDD		
$^{13}C_{12}$-1,2,3,7,8-P_5CDD		
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD		
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD		
$^{13}C_{12}$-OCDD		
$^{13}C_{12}$-2,3,7,8-TCDF		
$^{13}C_{12}$-1,2,3,7,8-P_5CDF		
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF		
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF		

Note: (1) Results are corrected for surrogate recovery.
(2) DL = detection limit (pg/L/analyte peak); NP = number of analyte peaks.
(3) * Value represents maximum possible amount as this isomer could coelute with other isomer(s).
(4) ND = not detected.
(5) NDR = not detected due to incorrect ratio.
(6) C = contaminant in corresponding method blank.
(7) E = possible chlorinated diphenyl ether interference.

Figure 8 Effluent Sample Data Sheet-Part I

Effluent Sample Data Sheet-Part II

Lab: _____ Final Volume: _____

Mill: _____ GC Column & MS: _____

Sample ID: _____ File: _____

Approved by: _____

Analyte	Ions Monitored	Ion Abundance Ratio	Acceptable Range for Abundance Ratio	Deviation from Expected Retention Time (sec)
2,3,7,8-TCDD			0.65 - 0.89	
1,2,3,7,8-P_5CDD			1.32 - 1.78	
1,2,3,4,7,8-H_6CDD			1.05 - 1.43	
1,2,3,6,7,8-H_6CDD			1.05 - 1.43	
1,2,3,7,8,9-H_6CDD			1.05 - 1.43	
1,2,3,4,6,7,8-H_7CDD			0.88 - 1.20	
OCDD			0.76 - 1.02	
2,3,7,8-TCDF			0.65 - 0.89	
1,2,3,7,8-P_5CDF			1.32 - 1.78	
2,3,4,7,8-P_5CDF			1.32 - 1.78	
1,2,3,4,7,8-H_6CDF			1.05 - 1.43	
1,2,3,6,7,8-H_6CDF			1.05 - 1.43	
2,3,4,6,7,8-H_6CDF			1.05 - 1.43	
1,2,3,7,8,9-H_6CDF			1.05 - 1.43	
1,2,3,4,6,7,8-H_7CDF			0.88 - 1.20	
1,2,3,4,7,8,9-H_7CDF			0.88 - 1.20	
OCDF			0.76 - 1.02	
$^{13}C_{12}$-2,3,7,8-TCDD			0.65 - 0.89	
$^{13}C_{12}$-1,2,3,7,8-P_5CDD			1.32 - 1.78	
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD			1.05 - 1.43	
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD			0.88 - 1.20	
$^{13}C_{12}$-OCDD			0.76 - 1.02	
$^{13}C_{12}$-2,3,7,8-TCDF			0.65 - 0.89	
$^{13}C_{12}$-1,2,3,7,8-P_5CDF			1.32 - 1.78	
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF			0.43 - 0.59	
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF			0.88 - 1.20	

Figure 8 Effluent Sample Data Sheet-Part II

Table 5 Target Method Detection Limits for Pulp Mill Effluents

Analytes	MDL (pg/L)*
TCDD/TCDF	2 - 5
P_5CDD/P_5CDF	4 - 10
H_6CDD/H_6CDF	4 - 10
H_7CDD/H_7CDF	6 - 15
OCDD/OCDF	8 - 20

* For individual analyte peaks including 2,3,7,8-substituted congeners.

Section 7

Data Reporting

Section 6 of the *Pulp and Paper Mill Effluent Chlorinated Dioxins and Furans Regulations* requires that operators take samples of the final effluent and the combined bleach plant effluent(s) according to Schedules I and II. The sample collection and compositing procedures for this are described in Appendix A.

The concentrations of substances set out in Schedule III of the regulations are to be measured in accordance with this reference method, and the results reported to Environment Canada. In addition, the information specified in the following paragraphs must also be submitted. Reporting shall be in writing or in an electronic format, where the electronic format is provided by Environment Canada.

Submission of sample results must be accompanied by a clear photocopy of the corresponding sample submission/custody form. An example of the sample submission/custody sheet is given in Figure 9.

For every sample analyzed, information on sample identity, sample size, GC column, concentration (pg/L) of target analytes, detection limits, number of isomer peaks identified for each homologue, amounts of surrogates added, and surrogate recoveries must be reported as shown in Figure 8, Part I.

Ion abundance ratio and relative retention time data must be reported for all 2,3,7,8-substituted congeners, as shown in Figure 8, Part II.

QA/QC data that must be submitted with results include results of the method performance tests (Figure 10), multi-point calibration data (Figure 11), calibration verification data (Figure 12), GC/MS detection limits verification data (Figure 13) and method blank results.

All original sample and supporting QA/QC data must be archived on disk or tape for a minimum of three years for possible audit. This would include glassware proof rinse data and results from GC Column Performance and Window Defining Mixture verification runs. Copies of ion chromatograms showing isotope abundance ratios and check marks for positive analyte peaks, lists of integrated peak areas and retention times for all monitored ions, and the date and time of sample analysis must be provided to Environment Canada upon request, as well as hard copy verification of MS resolution.

Sample Submission/Custody Sheet

Date: _____ Submitted by: _____

Mill: _____ Phone No: _____

Submitted to: _____ Fax No: _____

Storage Conditions During Shipment: _____

Sample ID	Date & Time Collected	Sampling Location	Matrix & Size	Container Type & Size	Analysis Requested
Remarks:					

CHAIN OF CUSTODY RECORD				
Relinquished by:	Date: / Time:	Received by:	Date: / Time:	Remarks:
Relinquished by:	Date: / Time:	Received by:	Date: / Time:	Remarks:
Relinquished by:	Date: / Time:	Received by:	Date: / Time:	Remarks:
Relinquished by:	Date: / Time:	Received by:	Date: / Time:	Remarks:

Figure 9 Sample Submission/Custody Sheet

Method Performance Test Data Sheet

Lab: _____ GC Column & MS: _____

Project: _____ Calibration Date: _____

Sample Matrix & Size: _____ Analysis Date: _____

Sample ID: _____ File: _____

Approved by: _____

Analyte	Amount Added pg	% Recovery #1	#2	#3
2,3,7,8-TCDD				
1,2,3,7,8-P_5CDD				
1,2,3,4,7,8-H_6CDD				
1,2,3,6,7,8-H_6CDD				
1,2,3,7,8,9-H_6CDD				
1,2,3,4,6,7,8-H_7CDD				
OCDD				
2,3,7,8-TCDF				
1,2,3,7,8-P_5CDF				
2,3,4,7,8-P_5CDF				
1,2,3,4,7,8-H_6CDF				
1,2,3,6,7,8-H_6CDF				
2,3,4,6,7,8-H_6CDF				
1,2,3,7,8,9-H_6CDF				
1,2,3,4,6,7,8-H_7CDF				
1,2,3,4,7,8,9-H_7CDF				
OCDF				

Surrogate	Amount Added pg	% Recovery #1	#2	#3
$^{13}C_{12}$-2,3,7,8-TCDD				
$^{13}C_{12}$-1,2,3,7,8-P_5CDD				
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD				
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD				
$^{13}C_{12}$-OCDD				
$^{13}C_{12}$-2,3,7,8-TCDF				
$^{13}C_{12}$-1,2,3,7,8-P_5CDF				
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF				
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF				

Figure 10 Method Performance Test Data Sheet

Multi-point Calibration Data Sheet

Lab: _____ GC Column & MS: _____

Mill: _____ File: _____

Calibration Date: _____ Approved by: _____

	Relative Response Factor (RRF)				Mean	%RSD
Analyte	CS1	CS2	CS3	CS4	RRF	
2,3,7,8-TCDD						
1,2,3,7,8-P_5CDD						
1,2,3,4,7,8-H_6CDD						
1,2,3,6,7,8-H_6CDD						
1,2,3,7,8,9-H_6CDD						
1,2,3,4,6,7,8-H_7CDD						
OCDD						
2,3,7,8-TCDF						
1,2,3,7,8-P_5CDF						
2,3,4,7,8-P_5CDF						
1,2,3,4,7,8-H_6CDF						
1,2,3,6,7,8-H_6CDF						
2,3,4,6,7,8-H_6CDF						
1,2,3,7,8,9-H_6CDF						
1,2,3,4,6,7,8-H_7CDF						
1,2,3,4,7,8,9-H_7CDF						
OCDF						

Figure 11 Multi-point Calibration Data Sheet

Calibration Verification Data Sheet

Lab: _____ GC Column & MS: _____

Mill: _____ Verification Date & Time: _____

Calibration Date: _____ File: _____

Standards: _____ Approved by: _____

Analyte	Ion Abundance Ratio	Calculated Value pg/µL	Actual Value pg/µL	Calculated Value / Actual Value
2,3,7,8-TCDD			5	
1,2,3,7,8-P_5CDD			10	
1,2,3,4,7,8-H_6CDD			10	
1,2,3,6,7,8-H_6CDD			10	
1,2,3,7,8,9-H_6CDD			10	
1,2,3,4,6,7,8-H_7CDD			10	
OCDD			20	
2,3,7,8-TCDF			5	
1,2,3,7,8-P_5CDF			10	
2,3,4,7,8-P_5CDF			10	
1,2,3,4,7,8-H_6CDF			10	
1,2,3,6,7,8-H_6CDF			10	
2,3,4,6,7,8-H_6CDF			10	
1,2,3,7,8,9-H_6CDF			10	
1,2,3,4,6,7,8-H_7CDF			10	
1,2,3,4,7,8,9-H_7CDF			10	
OCDF			20	

	Calculated % Recovery
$^{13}C_{12}$-2,3,7,8-TCDD	
$^{13}C_{12}$-1,2,3,7,8-P_5CDD	
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD	
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD	
$^{13}C_{12}$-OCDD	
$^{13}C_{12}$-2,3,7,8-TCDF	
$^{13}C_{12}$-1,2,3,7,8-P_5CDF	
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF	
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF	

Figure 12 Calibration Verification Data Sheet

GC/MS Detection Limits Verification Data Sheet

Lab: _____ GC Column & MS: _____

Mill: _____ Verification Date & Time: _____

Calibration Date: _____ File: _____

Standards: _____ Approved by: _____

Analyte	Ion Abundance Ratio	Calculated Value pg/µL	Actual Value pg/µL	Calculated Value / Actual Value
2,3,7,8-TCDD			0.25	
1,2,3,7,8-P_5CDD			0.50	
1,2,3,4,7,8-H_6CDD			0.50	
1,2,3,6,7,8-H_6CDD			0.50	
1,2,3,7,8,9-H_6CDD			0.50	
1,2,3,4,6,7,8-H_7CDD			0.50	
OCDD			1.0	
2,3,7,8-TCDF			0.25	
1,2,3,7,8-P_5CDF			0.50	
2,3,4,7,8-P_5CDF			0.50	
1,2,3,4,7,8-H_6CDF			0.50	
1,2,3,6,7,8-H_6CDF			0.50	
2,3,4,6,7,8-H_6CDF			0.50	
1,2,3,7,8,9-H_6CDF			0.50	
1,2,3,4,6,7,8-H_7CDF			0.50	
1,2,3,4,7,8,9-H_7CDF			0.50	
OCDF			1.0	

	Calculated % Recovery
$^{13}C_{12}$-2,3,7,8-TCDD	
$^{13}C_{12}$-1,2,3,7,8-P_5CDD	
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD	
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD	
$^{13}C_{12}$-OCDD	
$^{13}C_{12}$-2,3,7,8-TCDF	
$^{13}C_{12}$-1,2,3,7,8-P_5CDF	
$^{13}C_{12}$-1,2,3,4,7,8-H_6CDF	
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDF	

Figure 13 GC/MS Detection Limits Verification Data Sheet

Quality Assurance Summary

Required quality assurance elements of this method are summarized as follows:

(a) Before any effluent samples are processed, all pre-cleaned glassware, including Soxhlet apparatus, concentrators, columns, flasks, and vials, are rinsed with dichloromethane and hexane. Rinses are combined and processed in the same manner as test samples. Contamination levels of individual 2,3,7,8-substituted tetra, penta, hexa, hepta and octa CDD/CDF congeners in glassware proof rinses must not exceed 5,10,10,15 and 50 pg per sample, respectively.

(b) Before any effluent samples are processed, laboratory capability must be demonstrated by conducting triplicate analyses of matrix blanks (purified water) spiked with native and labelled PCDD/PCDF standards. Criteria for accuracy and surrogate recoveries described in Subsection 5.2 must be met.

(c) Before extraction, each sample is spiked with a mixture of isotopically-labelled surrogates to assess the degree of analyte loss during sample workup. If the recovery of any surrogate is outside the range of 40 to 130% for TCDD and TCDF, and 30 to 130% for penta, hexa, hepta and octa CDDs/CDFs, the sample must be re-processed and re-analyzed.

(d) Known concentrations of $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-H_6CDD must be added to each sample extract immediately before GC/MS analysis. These two compounds serve as retention time references for labelled surrogates and as the basis for calculation of surrogate recoveries.

(e) A method blank sample, consisting of 1 L of high purity water spiked with surrogates, is processed with each batch of up to 10 test samples. Acceptance limits for 2,3,7,8-substituted dioxin and furan congener presence in method blank samples are: 5 pg for TCDD and TCDF; 10 pg for penta and hexa CDD/CDF; 15 pg for hepta CDD/CDF; 50 pg for OCDD/OCDF. A compliance sample result for a 2,3,7,8- substituted congener must be flagged with a "C" if the same congener was present in the corresponding method blank at a level exceeding the applicable acceptance limit.

(f) Hard copied verification of MS resolution at 10 000 or better is required before and after each series of injections related to the application of this method.

(g) A Window Defining Mixture containing the first and last eluting isomer within each homologous group of PCDDs/PCDFs must be used to correctly define retention time windows for selected ion monitoring of individual homologues. This analysis must be repeated daily (see Subsections 6.2 and 6.3).

(h) **Acceptable chromatographic separation between 2,3,7,8-TCDD and its closest neighbouring isomers must be confirmed daily. If analysis for 2,3,7,8- TCDF on a second column is required, acceptable chromatographic separation between this isomer and its closest neighbouring isomers must also be demonstrated.** (See Subsection 6.3.)

(i) **Before sample analysis, calibration curves are constructed to verify linearity of MS response for all homologues over the concentration range of 0.25 to 100 pg/μL for native PCDDs/PCDFs** (see Subsection 6.6).

(j) **The established calibration must be verified by analyzing the calibration verification standard (CS3) at least once during every 12-hour period in which sample analysis occurs. Using four-point average RRFs obtained from initial calibration runs, the calculated concentrations of both 2,3,7,8-TCDD and 2,3,7,8-TCDF must be within 15% of their actual values. The calculated concentrations of all other native analytes must be within 20% of their respective true concentrations. The calculated recovery of each surrogate compound must be within the range of 75 to 125%. Remedial action is required whenever any native or surrogate compound fails this verification test (see Subsections 6.4 and 6.6).**

(k) **Gas chromatograph/mass spectrometer (GC/MS) detection limits must be assessed by analyzing the lowest concentration (CS1) standard solution. This analysis must be repeated daily.**

(l) **As a check on accuracy, NIST (formerly NBS) Reference Material 1614 (2,3,7,8-TCDD in solution) is periodically analyzed as a sample. This aspect of the QA program should be enhanced by the use of other reference materials, as they become available.**

(m) **Reported sample results must be fully documented. All QA/QC documentation and raw GC/MS data, must be available for auditing** (see Section 7).

Section 9

Method Validation

Studies are currently under way to assess the precision and accuracy of this method. A report on these studies will be published separately.

References

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Appendix A

Sample Collection Protocol

1.0 Introduction

It is important to ensure that representative samples are collected which are free from outside contamination, and are properly preserved. The following is proposed as a means of collecting representative samples, and minimizing sample contamination and degradation.

Two 1-L composite samples should be collected for each final effluent and combined bleach plant effluent as defined in the following sections and at the frequencies specified in Schedules I and II of the *Pulp and Paper Mill Effluent Chlorinated Dioxins and Furans Regulations*. These samples may be prepared by using either manual or automatic sampling techniques as described in Section 3 of this Appendix.

2.0 Sample Contamination

There is the potential for contamination in every step of collection, preservation, shipping, and handling of dioxin/furan samples. Some sources of potential contamination include improperly cleaned sample containers, tubing, etc., poor handling, not using or improper use of latex gloves, and dirty conditions at sampling sites. Amber glass bottles with Teflon-lined caps are the best sample containers for dioxin/furan samples. Samples should never come into contact with plastic other than Teflon, except at autosampler pump heads where minimal lengths of surgical silicone tubing are acceptable. All sample containers (bottles, graduates, etc.) should be washed with standard laboratory-grade detergent, followed by rinsing with aliquots of deionized water, acetone, hexane, and dichloromethane. Containers should be air-dried in a contaminant-free area and then capped or foil-covered before use. Precleaned and proofed bottles are commercially available.

It is recommended that all sampling devices and equipment, their containers and all tubing, valves and contact components be dedicated to a particular sampling site in order to minimize the possibility of cross-contamination. It is the user's responsibility to demonstrate that the sampling equipment is clean, free from contamination, and suited for sampling and analysis needs. Generally, the cleaning and preparation of any relocated equipment should include hot water, phosphate-free detergent washing, hot water rinse, acetone rinse, hot and cold water rinses, distilled water rinse and, finally, multiple rinses with the actual effluent being sampled. Contact openings of cleaned equipment should be foil-covered in transit.

It is also recommended that new non-talc latex gloves be put on just before handling the sample aliquot bottle at each site, after any dirty work is completed. This precaution minimizes opportunity for cross-contamination.

3.0 Sampling of Final Effluent

Final effluent samples should be collected in the stream discharging to receiving waters, downstream of all on-site wastewater treatment devices. If there are multiple

points of discharge, then each discharge stream must be sampled, to be composited later into a single final effluent. Samples can be collected by either a manual or automated method. With either method, it is essential to collect at a point of thorough mixing.

3.1 Manual Sampling Method

The sample can be collected by using simple field equipment, including buckets, funnels, and suitable lengths of chain or dip poles. The equipment must be suited to the sampling and analysis being performed, and should generally be dedicated to the sampling site. Special procedures for cleaning of relocated equipment are defined in Section 2 of this Appendix.

This approach is less rigorous than continuous automated sampling, and requires manual sampling of the effluent stream at regular intervals over a 24-hour period, in order to obtain a representative sample.

Manual sampling can be conducted using an automated sampler in manual mode in cases of automatic mode failure.

3.1.1 Detailed Sample Collection Procedure

The sample shall be obtained by taking manual grab samples in precleaned 500-mL aliquot bottles at a frequency of one grab sample at least every three hours (over a 24-hour period) from a representative point in the final effluent stream.

The following procedure should be undertaken during sample collection.

a) Open sampling valves and/or sewer covers.

b) Put on latex gloves.

c) Remove cap from a precleaned aliquot bottle.

d) Place cap liner up on a surface away from potential sources of contamination.

e) Place the bottle in a reaching-assist device (if necessary) and place in the flow. If not possible, use a pre-washed, stainless steel bucket to collect the sample.

f) Rinse the aliquot bottle (or bucket) twice with the sample to be collected.

g) Fill the aliquot bottle with the sample.

h) Rinse the cap by pouring about 10% of the contents of the aliquot bottle over the cap.

i) Refill the aliquot bottle to the top.

j) Place the bottle on a solid surface.

k) Screw the cap onto the aliquot bottle.

l) Identify all aliquot bottles as to their contents, location and time of sampling.

m) Place the aliquot bottle into a cooler (kept at 1 to 5°C).

n) Close all sampling valves and sewer covers.

o) At the end of the 24-hour period, transport the aliquot bottles to the laboratory in the cooler.

p) From each aliquot bottle, transfer the required volume [see (q)] into each of two precleaned 1-L sample bottles, as follows:

- rinse a 500-mL precleaned graduated cylinder, specific for the effluent stream;
- for rinsing, shake the aliquot bottle and pour 10 mL into the 500-mL

graduated cylinder. Ensure *all* inside surfaces are thoroughly rinsed. Pour the rinsing volume out, and repeat the rinse with another 10 mL;

- shake the aliquot bottle again and pour the required volume [see (q)] into the 500-mL graduated cylinder;
- pour this volume into one of the 1-L sample bottles; and,
- repeat the shake and pour sequence for the second 1-L bottle.

q) The required volume from each aliquot bottle is 1 L divided by the number of aliquot bottles (for a time-proportional sample). For a flow-proportional sample, this volume is multiplied by the ratio: flow at aliquot collection/24-hour average flow.

r) Tightly cap both 1-L bottles with precleaned Teflon caps, and identify as to contents, location and date.

3.2 Autosampling Method

Final effluent samples may be collected by using automated sampling equipment. The autosamplers must be mechanically and electrically suited to the environment in which they will serve, and should be easily accessible for routine inspection and maintenance. Ability to obtain a representative sample, material composition, and temperature stability are the three most important characteristics of automated sampling equipment.

It is essential that the autosampler take its sample from a location in the final effluent stream that will provide a representative point of thorough mixing. The material composition characteristics can generally be met through consistent use of materials such as Teflon and glass. Minimal lengths of surgical grade silicone rubber tubing may be used at peristaltic pump heads.

The temperature stability is best monitored with a continuous temperature recorder and documented in a sampler-specific log book that is kept close to the sampler. This log book should also incorporate repair, inspection, routine use, and maintenance records.

The detailed automatic sampling procedure recommended by the automatic sampling equipment suppliers should be followed.

Two 1-L 24-hour composite samples of the final effluent shall be collected during a period of 24 hours by:

a) taking a continuous sample of the final effluent at a constant or flow-proportional rate;

b) compositing final effluent aliquots of equal volume (taken at least every three hours); or,

c) compositing final effluent aliquots of equal volume (taken at least every three hours) in a quantity proportional to flow at the time of aliquot collection.

The autosampler should be programmed, if possible, to utilize the purge function. It should produce sufficient or more than sufficient volume at the end of 24 hours. If it fills a single container only, the sample should be split into two 1-L sample bottles at the end of the sampling period, while stirring with a precleaned glass rod, leaving a slight headspace. If it fills one or more series of separate aliquot bottles, transfer to two 1-L sample bottles as described in Subsection 3.1.1(p), or by direct shaking and pouring if volumes are equal to those required.

3.3 Compositing of Multiple Discharges

If there are multiple discharges, separately sampled, then a flow-proportional composite sample must be prepared. The method described in Subsection 4.3 of this Appendix is followed to produce two 1-L composite sample bottles.

4.0 Sampling of Combined Bleach Plant Effluent

A combined bleach plant sample is produced for each bleach plant. It includes all seal tank overflows that leave the bleach plant, excluding any recycle flows that are reused within the bleach plant. It also excludes any lime mud or other additions from outside the bleach plant. It will usually be necessary to obtain the sample manually, as a flow-proportional composite of acid and alkaline filtrates or acid and alkaline sewers. Results should be reported with associated total effluent flows for each bleach plant.

4.1 Manual Sampling

4.1.1 Detailed Sample Collection Procedure

In the case of acid sewer or acid bleach filtrates, the following additional procedure should be taken for each sampled stream.

a) Before beginning sampling of each aliquot: obtain the residual chlorine reading from the bleach plant operator.

b) The procedure (j) as described in Subsection 3.1.1 should be followed by: adding Na_2SO_3, proportional to the amount shown in Table A.1 (based on the residual chlorine reading) to the aliquot bottle.

4.2 Automatic Sampling

Procedures follow those described in Subsection 3.2, with the additional requirement for acid streams of pre-charging autosampler bottles with Na_2SO_3. The amount is determined from Table A.1, considering the autosampler bottle volume and the expected limit of chlorine residual.

Table A.1 Sodium sulphite requirements for acid sewer or acid bleach filtrate aliquot bottles

Chlorine Residual (mg/L)	Amount of Na_2SO_3 Required for Each 500-mL Aliquot Bottle (mg)
1 - 100	95
101 - 200	191
201 - 300	287
301 - 400	382
401 - 500	478

Note: The Na_2SO_3 will be added to the 500 mL aliquot in the field (at the time of sample collection).

4.3 Compositing Techniques for Bleach Plant Effluent

The combined bleach plant effluent sample is prepared by flow-proportional compositing of the individual effluent samples. Flows for the individual bleach plant effluent streams may be estimated by mass balance. The flow data for each effluent shall be obtained before preparing the final composite at the end of the 24-hour period.

The procedure for flow-compositing at the end of the 24-hour sampling period is applied to each of the two 1-L samples as follows.

a) Rinse a 500-mL precleaned graduated cylinder specific for each composite required.

b) For rinsing, shake the 1-L bottle representing the largest flow, and pour 10 mL into the 500-mL graduated cylinder. Ensure *all* inside surfaces are thoroughly rinsed. Pour the rinsing volume out, and repeat the rinse with another 10 mL.

c) Shake this bottle again and then pour the required volume (see Note 1) into the 500-mL graduated cylinder.

d) Pour this volume into the precleaned 1-L composite sample bottle.

e) Repeat the compositing procedure with all remaining samples contributing to the final composite.

f) Tightly cap the composite bottle, with a precleaned Teflon cap, and identify as to contents, location and date.

Note 1: The required volume to be retained from each contributing effluent is 1 L times the ratio: average flow of contributing effluent/total of average flows for all contributing effluents.

5.0 Shipping and Storage of Samples

All samples should be kept at 1 to 5°C during collection and shipping to minimize the potential for degradation. Shipping coolers will be equipped with a suitable number of ice packs to ensure that the samples are kept cold. Individual bottles in the cooler should be separated by protection sleeves and any large empty spaces in the cooler should be filled. Automatic samplers should have their own cooling system.